change of EF1 play a key role of regulation.

Keywords: plants, EF-hand proteins, GTP-binding proteins

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Crystal structure and functional study of wild type and mutated *Bacillus cereus* NCTU2 chitinase

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Chitinases which hydrolyze chitin as carbon and nitrogen nutrient, occur in a wide range of organisms include in viruses, bacteria, fungi, insects, higher plants, and animals. Agene of family 18 chitinase from *Bacillus cereus* NCTU2 encodes a signal peptide (27amino acids) and a mature protein (333 amino acids), The gene of family 18 chitinase from *Bacillus cereus* NCTU2 was overexpressed by *E. coli* BL21 (DE3) strain. ChiNCTU2 and mutant E145Q of MW 36 kDa have been crystallized using the hanging-drop vapor diffusion method with solution consisted of polyethene glycerol 8000, sodium cacodylate and zinc acetate dihydrate. According to diffraction of ChiNCTU2 crystals at resolution 1.20 Å, the unit cell belongs to space group $P2_1$ and has parameters a = 50.789 Å, b = 48.788 Å and c = 66.867 Å. And E145Q crystal at resolution 1.49, the unit cell belongs to space group $P1$ and has parameters a = 61.306 50.820 Å, b =72.888 Å and c = 76.343 Å. The protein structure of ChiNCTU2 is monomer by using multiwavelength anomalous dispersion method and the crystal packing of E145Q is tetramer by using molecular replacement method. Four residues Asp143, Glu145, Glu190 and Gln225 bind with zinc atoms in the catalytic domain of ChiNCTU2 protein structure. We proved that zinc atoms decline activity of ChiNCTU2 by detecting the amount of chitobioside using DNS (3,5-Dinitrosalicylic acid). According to structure and mutagenesis we found that E145, Q225 and Y227 are the most important redidues for its function.

Keywords: chitin, chitinase, structure

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The structure of human diamine oxidase

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The crystal structure of human diamine oxidase (hDAO), the first reported structure of a diamine oxidase (DAO), has been determined to 2.9 Å resolution. DAO, a copper-containing amine oxidase (CuAO), contains a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor derived by post-translational modification of a tyrosine residue [1]. DAO is distinguishable among members of the CuAO enzyme family in its ability to oxidize diamines, such as putrescine and cadaverine, as well as monoamines. DAO is involved in many biological processes. In mammals DAO is found in several tissues, with the highest reported expression levels found in the placenta, small intestine and kidneys. In particular, hDAO may play an important role in histamine metabolism (1). We have grown orthorhombic crystals of hDAO belonging to the space group $C222_1$, with unit-cell dimensions a=95.0, b=97.2, c=179.2 Å. These crystals diffracted to 2.9 Å in-house at 100 K. Data were integrated and scaled with the HKL suite of programs, DENZO and SCALEPACK. The data is 98.3% complete in the range 50-2.9 Å with an overall R_{merge} of 8.4%. The most reasonable Matthews' coefficient suggests there is one molecule in the asymmetric unit with 40% solvent content using 100 kDa as the molecular mass. The structure was solved by molecular replacement, PHASER v1.3 giving a Z-score of 26.2 with a search model created using CHAINSAW, with human vascular adhesion protein-1 (hVAP-1, PDB code 1US1) as the target. Initial rigid-body and restrained refinement has been carried out using REFMAC v5.2. 2*Fo-Fc* and *Fo-Fc* electron-density maps were inspected with, and modeled using COOT.

[1] Elmore, B. O., Bollinger, J. A., and Dooley, D. M. (2002) *J Biol Inorg Chem* **7**(6), 565-579

Keywords: diamine oxidase, amine oxidase, topaquinone

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A study of protocatechuate 3,4-dioxygenase muntants and substrate interactions

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Protocatechuate 3,4-dioxygenase is a nonheme, iron containing enzyme that catalyzes the intradiol oxidative cleavage of 3,4-dihydroxybenzoic acid to ß-carboxy-*cis*,*cis*-muconic acid via incorporation of molecular oxygen into the aromatic ring of the substrate. In an attempt to further understand the factors involved in substrate turnover and mechanism, a series of second sphere residue mutants has been created and structurally and kinetically examined. These crystals diffract to high resolution and show clearly that alterations of these second sphere residues can dramatically affect the interactions with substrate and substrate analogs. A detailed structural and kinetic comparison of these mutants will be presented.

Keywords: structure and function, structural enzymology, metalloenzymes

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Structural study of H_2O_2 reductase, rubperoxin

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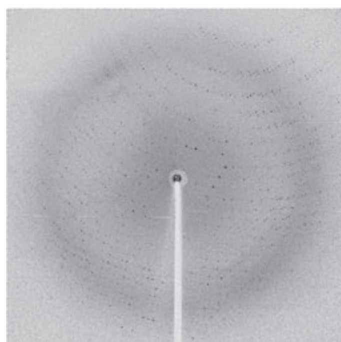
Rubperoxin (Rpr) was identified as an O_2-induced protein in

Clostridium acetobutylicum, an obligatory anaerobe. Rpr consists of 181 amino acids with a molecular weight of 22,500. It is rubrerythrin-like protein, and conserves rubredoxin-type [Fe(SCys)$_4$] site in the N-terminus. Rpr rapidly reduces hydrogen peroxide using NADH as an electron donor, and slowly reduces t-butyl hydroperoxide and dioxygen. $2H^+ + 2Rpr_{red}(1Rpr_{dimer\ red}) + H_2O_2 = 2Rpr_{ox}(1Rpr_{dimer\ ox}) + 2H_2O$ In order to reveal the reaction mechanism of the reduction of hydrogen peroxide by Rpr, we have started the crystallographic study. Purified Rpr was crystallized by the sitting-drop vapor diffusion method using poly(ethylenglycol)(PEG) 4000 as a precipitant (final composition of the droplet: 12 mg/mL in 50 mM K-Pi pH 7.0 buffer, Outer droplet: 9% PEG 4000, 0.1 M Zn acetate in 0.1 M MES pH 6.0 buffer). Crystals diffracted to 2.3 angstrom resolution at the beamline BL38B1 (SPring-8), and belong to the space group $P2_12_12$ (a = 81.6 Å, b = 117.4 Å, c = 141.5 Å). Structure analysis is now in progress.



Keywords: X-ray crystallography of proteins, metalloproteins, redox enzymes

P04.03.180

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Roles of heme-6-propionate side chain in monooxygenase cytochrome P450cam

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Cytochrome P450cam (P450cam) responsible for *d*-camphor hydroxylation binds a protoheme IX as a prosthetic group in which two heme-propionate side chains interact with Arg112 and Arg299, respectively. To understand the structural and functional roles of the heme-6-propionate side chain in P450cam, we prepared the reconstituted P450cam with one-legged heme where the 6-propionate side chain was replaced with methyl group. The crystal structure of the reconstituted P450cam at a resolution of 1.55 angstrom is highly superimposable with that of the wild type protein, and a chloride anion is located in the position of the 6-propionate (Figure 1). The reactivity of the reconstituted P450cam toward the substrate hydroxylation was almost comparable with that observed for the wild type protein, whereas we found that the removal of the 6-propionate side chain accelerated the unfavorable conversion of the P450 active form into the inactive P420 species. In this presentation, we discuss the exact roles of the 6-propionate side chain in P450cam.

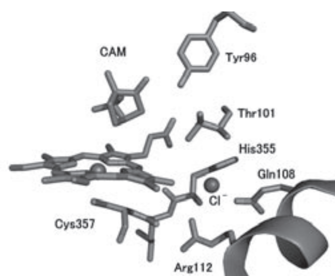

Figure 1. The crystal structure of the surrounding of the one-legged heme of the reconstituted P450cam.

[1] K. Harada, K. Sakurai, H. Shimada, T. Hayashi *et al.* (2007) *J. Am. Chem. Soc.*, **130**, 432.

Keywords: heme proteins, monooxygenases, crystallography of biological macromolecules

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The crystal structure of heme oxygenase catalytic intermediate unravel the enzyme mechanism

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Heme oxygenase (HO) catalyzes O_2-dependent regiospecific conversion of heme to biliverdin, CO, and free iron by three consecutive monooxygenase steps. In order to understand the reaction mechanism of HO, we have solved the structures of catalytic intermediate of HO catalysis using HmuO, a heme oxygenase from *Corynebacterium diphtheriae*. In the substrate-free structure, the proximal and distal helices, which sandwich the heme group, move farther apart with changes in their conformations resulting in opening of the heme pocket so as to fascilitate heme binding. Crystals of the hydroperoxo intermediate, have been prepared using cryogenic reduction technique, in which the oxy crystals were irradiated by synchrtron radiation at 100 K. The structure of the ferric hydroperoxo heme-HmuO complex is very similar to that of the oxy form but has slightly longer Fe-O and O-O bond distances. Flash-annealing of the irradiated oxy crystals has yielded the hydroxyheme intermediate, the structure of which has been solved and refined at high resolution. In the structure, the distal helix moves away from hydroxyheme. Possible reaction mechanism, based on the crystal structures of catalytic intermediates will be discussed at the meeting.

Keywords: heme enzyme structure and function, reactive intermediate, cryo crystallography

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X-ray crystal structural analysis of cyanide binding cytochrome *c* oxidase

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For elucidation of the reaction mechanism of the cyotochrome *c* oxidase(CcO), it is desirable to determine the binding mechanism of cyanide to the oxygen reduction site of the enzyme. Here, we analyzed the structure of the cyanide derivative of the fully oxidized CcO. Cyanide induces extremely small change in the α-band spectrum of the enzyme. Thus, it is impossible to trace cyanide-binding to the enzyme in the crystals by measuring the absorption spectrum of the crystals, because accurate measurement of Soret-band of the enzyme in crystals is impossible. However, we found that cyanide once bound to CcO was not removal by repeat dialysis,